

FINAL REPORT

PARASITE, PREDATOR, AND DISEASE MANAGEMENT
STRATEGIES IN ALFALFA LEAFCUTTING BEES

19970004

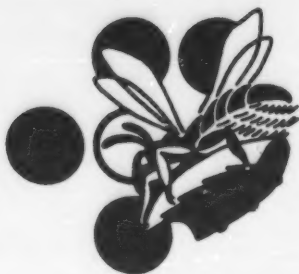
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Canada¹

Saskatchewan



SASKATCHEWAN ALFALFA SEED PRODUCERS' ASSOCIATION

RESEARCH TECHNICAL REPORT

**AFIF-Hub PROJECT 19970004
FINAL REPORT**

**Parasite, predator, and disease management
strategies in alfalfa leafcutting bees**

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Table of Contents

A. Summary

B. Technical report

1. Background and objectives
2. Project activities and achievements
3. Research results and conclusions
 - a) Parasite control research initiatives
 - i. Laboratory research on alternative compounds for parasite control
 - ii. Laboratory research on aspects of chalcid re-parasitism
 - iii. Field level research on alternative compounds for parasite control
 - iv. Research on prevention of chalcid re-parasitism in the field
 - b) Chalkbrood disease research initiatives
 - c) Research on stored product pests in alfalfa leafcutting bee populations
 - d) 1997-2000 alfalfa leafcutting bee trapnest research summary
 - e) Alfalfa leafcutting bee winter survey summary (1997-1998 through 2000-2001)
4. Indications for further research

C. Personnel

D. Equipment rental

E. Project photos

F. AFIF acknowledgement

G. Technology transfer

I. Deliverables for evaluation

A. Summary

This objective of this research project has been to monitor parasite, predator, and disease levels in Saskatchewan alfalfa leafcutting bee populations and to develop management strategies which will assist in maintaining high quality bee populations in order to enhance alfalfa seed production and increase the value of the bees in export markets. Surveys undertaken during the 1997-2000 field seasons, and throughout the subsequent winter periods, have identified increasing parasite levels in some populations and several occurrences of chalkbrood disease. Research in the area of parasite control has involved work to develop a prototype leafcutting bee research incubator designed to enhance parasite control during spring bee incubation, and work to further develop the use of pyrethrin aerosols for parasite control. Research has also been undertaken to identify stored product pests associated with bee populations, and to develop practical methods for dealing with these pests.

B. Technical report

1. Background and objectives

The alfalfa leafcutting bee, *Megachile rotundata*, is an important pollinator of alfalfa for seed production and has been used by Saskatchewan alfalfa seed producers as a dependable pollinator for about thirty years. Alfalfa seed production in the province has expanded rapidly over the past several years to the current level of approximately 100,000 acres, representing 75% of the alfalfa seed acreage in Canada. Alfalfa leafcutting bee populations, estimated at 20,000 per acre, have grown to a total of approximately 2 billion. Under ideal conditions, this population doubles each year and the excess production is marketed primarily to alfalfa seed growers in the United States. Saskatchewan alfalfa leafcutting bee sales currently total \$10 - \$15 million each year.

Infestations of the chalcid parasite (*Pteromalus venustus*) are currently a major problem in Saskatchewan alfalfa leafcutting bee populations. Another factor which may limit alfalfa leafcutting bee production is chalkbrood disease (*Ascosphaera aggregata*) and the occurrence of a related fungal pathogen (*Ascosphaera larvis*) which has recently been identified in Saskatchewan alfalfa leafcutting bee populations. Several stored product pest insect species with the potential to severely damage Saskatchewan alfalfa leafcutting bee populations have also been identified.

This specialized alfalfa leafcutting bee research project is designed to evaluate parasite, predator, and disease levels in Saskatchewan alfalfa leafcutting bee populations through extensive monitoring of the quality of these bee populations, to identify potential problems in these areas, and to develop management strategies which will assist alfalfa seed producers in maintaining high quality alfalfa leafcutting bee populations. This will serve to enhance alfalfa seed production in Saskatchewan and will also increase the value of alfalfa leafcutting bees in export markets.

B. Technical report

2. Project activities and achievements

Research undertaken over the four period 1997-2000 on alfalfa leafcutting bee parasites, insect pests, and diseases has involved extensive monitoring of Saskatchewan alfalfa leafcutting bee populations during each field season through the trapnest survey, laboratory and field initiatives in the area of chalcid parasite control, and laboratory research initiatives relating to chalkbrood disease. Baseline research on stored product pests associated with alfalfa leafcutting bee populations in Saskatchewan, first initiated during the 1997 field season, has been continued through the 1998-2000 field seasons; as well, extensive alfalfa leafcutting bee winter surveys have been undertaken annually in order to evaluate the quality of alfalfa leafcutting bee populations through analysis of bee cell samples solicited from alfalfa seed producers throughout the province.

The field season trapnest surveys and the alfalfa leafcutting bee winter surveys have assisted in the determination of chalcid parasite (*Pteromalus venustus*) levels in alfalfa leafcutting bee populations. The surveys were also essential for the identification of sites in the province where chalkbrood disease (*Ascosphaera aggregata*) is currently present in bee populations and made it possible to document the level of infection. Identification of chalkbrood is a key to the control of this devastating fungal disease. The trapnest and winter surveys have also served to provide research material for the investigation of a fungal disease related to chalkbrood (i.e. the ragged-brood species *Ascosphaera larvis*). Work undertaken on this pathogen in collaboration with investigators at the AAFC-Lethbridge research station in Alberta, involving a combined program of field and laboratory research on *Ascosphaera larvis*, resulted in the publication of a scientific manuscript on the potential for this fungal species to become a serious problem in alfalfa leafcutting bee populations.

The trapnest survey has been an important instrument as well for the collection of stored product pest insect specimens for subsequent rearing and identification in the laboratory. Work undertaken on this aspect of the research project marks the first research initiative ever undertaken to document stored product pests associated with alfalfa leafcutting bee populations in Saskatchewan.

Research has also continued to further develop pyrethrin aerosols for use as a chalcid parasite control agent. In order to assist in the development and transfer of this novel parasite control technology, another major research initiative was undertaken during the 1998-2000 field seasons with the purpose of designing and constructing a prototype alfalfa leafcutting bee research incubator. The prototype alfalfa leafcutting bee research incubator, which employs highly advanced engineering in air circulation and temperature control systems, has been utilized in the evaluation of pyrethrin aerosol compounds for chalcid parasite control. Development of the prototype alfalfa leafcutting bee incubator, incorporating features designed to optimize parasite control, will provide important technical information to alfalfa seed producers and assist in the construction of new alfalfa leafcutting bee incubators or in the retrofitting of existing incubators to a recommended set of specifications which will enhance parasite control efforts.

B. Technical report

3. Research results and conclusions

a) Parasite control research initiatives

The alfalfa leafcutting bee, *Megachile rotundata*, is an important pollinator of alfalfa for seed production. Infestations of the chalcid parasite, *Pteromalus venustus*, are a major problem in western Canadian populations of the alfalfa leafcutting bee. The chalcid parasite larvae in a parasitized alfalfa leafcutting bee cell develop to the adult stage so rapidly that multiple generations may occur in a relatively short period of time. This creates the potential for a significant increase in the parasite level within an alfalfa leafcutting bee population in a single season.

A research program has been undertaken over the past several years to identify compounds which may be efficacious for control of parasites in alfalfa leafcutting bee populations. Evaluation of a number of compounds has led to the identification of a pyrethrin aerosol spray compound as a promising parasite control agent. The pyrethrin is released into the incubator environment as an aerosol carrying small pyrethrin particles. Preliminary research has demonstrated that the most critical factor associated with use of pyrethrin aerosols for parasite control involves adequate circulation of the pyrethrin particles within the incubator.

Research to develop new technology for parasite control involves work in various areas of the alfalfa leafcutting bee system. Key time frames for parasite control include the spring incubation period, the summer field period, and the fall storage period. Development of successful parasite management strategies must address all of these areas of opportunity for re-parasitism. Research initiatives undertaken through during the period 1997 through 2000 have involved work at the laboratory level to determine safety of alternative compounds to the alfalfa leafcutting bee and efficacy of these compounds for parasite control, and research at the field level during the spring incubation period to determine alfalfa leafcutting bee safety and efficacy for parasite control with alternative compounds.

There is currently no standard alfalfa leafcutting bee incubator design utilized by alfalfa seed producers; this lack of uniformity makes development and transfer of novel parasite control technology difficult. For this reason, part of this research project involved an initiative to design and construct a prototype alfalfa leafcutting bee research incubator. The first prototype incubator to be constructed was utilized during the 1998 field season to further evaluate use of pyrethrin aerosol for chalcid parasite control. A second incubator of identical design was subsequently constructed and both incubators were utilized for chalcid parasite control research during the 1999 and 2000 field season. The purpose of research on the development of a prototype alfalfa leafcutting bee incubator, incorporating features designed to optimize parasite control, is to provide important technical information to producers which will assist them in chalcid parasite control efforts.

i. Laboratory research on alternative compounds for parasite control during the incubation period

In addition to work with pyrethrin aerosol compounds, several other compounds have been evaluated in a laboratory-scale research system for their efficacy as parasite control agents. These alternative compounds include GBX (composed of garlic extract - active ingredient allyl disulfide, an insect repellent agent), SPX (composed of spinosyns, a naturally-derived group of insect control molecules extracted from the bacterium *Saccharopolyspora spinosa*), and CO₂ gas.

Two of these alternative compounds, GBX and SPX, were initially tested on alfalfa leafcutting bee cells for their safety to developing and emerging leafcutting bees. Following these experiments on bee safety, a series of two-part tests were undertaken to evaluate the effect of these compounds on re-parasitism; in part I of these tests (choice component), parasites were given a choice of control or treated leafcutting bee cells, while in part II of these tests (no-choice component), parasites were placed on either control or treated leafcutting bee cells.

Tests of compound GBX (2.0% formulation) for bee safety indicated that adult bee emergence from GBX-treated cells was not significantly different than emergence from untreated (control) cells. In parasite control experiments, re-parasitism levels in cells treated with GBX (2% formulation) were as high as those observed in untreated (control) cells in both choice and no-choice tests (Table 1). Levels of pupal mortality due to chalcid parasite stinging were also high in both GBX-treated and untreated (control) cells, in choice and no-choice tests. These data indicate that GBX (2.0% formulation) was not efficacious for control of chalcid re-parasitism.

Table 1. Efficacy of GBX treatment for control of chalcid re-parasitism

Treatment	LCB emergence (%)	Cells re-parasitized (%)	Cells stung (%)
Part I (choice test)			
Control	20.5	47.9	16.4
GBX - 2.0%	35.2	39.4	15.5
Part II (no-choice test)			
Control	1.4	72.6	13.7
GBX - 2.0%	13.4	62.7	16.4

The alternative compound designated SPX was initially tested in 1998 on alfalfa leafcutting bee cells for its safety to developing and emerging leafcutting bees. Following experiments on bee safety, a series of two-part tests was undertaken to evaluate the effect of these compounds on re-parasitism; in part I of these tests (choice component), parasites were given a choice of control or SPX-treated leafcutting bee cells, while in part II of these tests (no-choice component), parasites were placed on either control or SPX-treated leafcutting bee cells. These tests of compound SPX undertaken in 1998 at two rates (50uL and 100uL) for bee safety indicated that adult bee emergence from SPX-treated cells was not significantly different than emergence from control cells. In parasite control (choice component) tests with SPX, levels of both re-parasitism and pupal mortality due to chalcid parasite stinging were visibly lower in cells treated with SPX. However, in the no-choice component of these SPX compound tests, levels of re-parasitism were actually higher in SPX-treated cells than in untreated (control) cells, though pupal mortality due to chalcid parasite stinging was lower in SPX-treated cells. These data indicated that although SPX appeared to be somewhat efficacious for control of chalcid re-parasitism, further work was required to determine if an effective cell treatment rate could be identified.

Research undertaken in 1999 involved testing of SPX compound at higher rates (100uL and 250uL). Once again, in addition to tests for bee safety, a series of two-part tests was undertaken to evaluate the effect of these higher rates on re-parasitism; protocols utilized in the parasite control tests were similar to those outlined above in 1998 tests, involving choice and no-choice components. Tests of compound SPX undertaken to evaluate 100uL and 250uL rates for bee safety indicated that adult bee emergence from SPX-treated cells was not significantly different from control cell emergence.

In parasite control (choice component) tests with SPX, parasites were given a choice of control or SPX-treated leafcutting bee cells (Table 2).

Table 2. Evaluation of the efficacy of SPX spray treatment for control of chalcid re-parasitism (choice test) - parasites introduced 7 days post-treatment

Treatment	LCB emergence (%)	Cells re-parasitized (%)	Cells stung (%)
Control	58.3	33.3	8.3
SPX - 100uL	61.4	38.6	0.0
SPX - 250uL	57.5	42.5	0.0

In these tests, the mean level of re-parasitized cells was actually higher in SPX-treated cells at both the 100uL and 250uL rates than in untreated (control) cells, though pupal mortality due to chalcid parasite stinging was lower in SPX-treated cells. Leafcutting bee adult emergence was not significantly different among treatments. In parasite control (no-choice component) tests with SPX, parasites were placed on either control or SPX-treated leafcutting bee cells (Table 3). In these tests, the mean level of re-parasitized cells was significantly lower in SPX-treated cells at both the 100uL and 250uL rates than in untreated (control) cells, while pupal mortality due to chalcid parasite stinging was slightly lower in cells treated with SPX. However, the level of re-parasitism was relatively high in all treatments, indicating that the SPX treatment did not provide the bee cells with the level of protection required in a field-scale alfalfa leafcutting bee incubation system.

Table 3. Evaluation of the efficacy of SPX spray treatment for control of chalcid re-parasitism (no-choice test) - parasites introduced 7 days post-treatment

Treatment	LCB emergence (%)	Cells re-parasitized (%)	Cells stung (%)
Control	21.1	76.3	2.6
SPX - 100uL	54.3	45.7	0.0
SPX - 250uL	66.7	33.3	0.0

Research to determine the efficacy for parasite control of various combined 30°C pre-incubation period / CO₂ gas treatment regimes utilized 100% CO₂ gas treatments of 24 or 48 hours duration following 30°C incubation of cells containing parasite larvae for periods of 1, 2, 3, 5, or 7 days. Following these treatments all cells were incubated at 30°C in order to determine percent parasite emergence and average time to emergence compared with untreated control cells containing parasite larvae.

Table 4. Mean parasitized cell percent emergence and time to emergence in control and CO₂-treated LCB cells

Treatment	Emergence (%)	Time to emergence (days)
Control	94.00	12.30
24 h CO ₂		
@ day 01	96.00	13.25
@ day 02	92.00	13.57
@ day 03	84.00	13.43
@ day 05	86.36	13.63
@ day 07	91.67	13.00
48 h CO ₂		
@ day 01	96.00	14.08
@ day 02	96.00	14.70
@ day 03	92.00	14.69
@ day 05	87.50	15.65
@ day 07	88.00	14.86

Data presented in Table 4 indicate that treatment with CO₂ gas following various 30°C pre-incubation periods had little or no effect on parasite emergence. Incubation of parasitized cells for up to 7 days does not render the developing chalcid parasite larvae susceptible to CO₂ gas-induced mortality under the conditions investigated in this study.

ii. Laboratory research on aspects of chalcid re-parasitism

The adult female chalcid parasite (*Pteromalus venustus*) typically stings the alfalfa leafcutting bee larva or pupa prior to ovipositing on the body of the host. During routine evaluation of unemerged alfalfa leafcutting bee cells from bee incubation trays during the 1997 field season, it was observed that in some cases, developing bee pupae were being stung and killed by chalcid parasites, but not successfully re-parasitized. This mortality due to stinging only was found at levels of up to 5.0% in a few bee populations having high levels of chalcid parasites.

In order to investigate the interaction between "successful re-parasitism" (i.e. stinging + oviposition) and "stinging only" in relation to the age of the larval or pupal host, an experiment was undertaken in which alfalfa leafcutting bee larvae and pupae at varying stages of development were exposed to adult female chalcid parasites. Control groups were left unexposed to adult female parasites in order to accurately assess maximum percent bee emergence in the population studied.

As noted in Table 5, while overall percent "successful re-parasitism" occurred at relatively uniform levels (range 5.3-14.1%) in larval and pupal bees at stages from 1 - 17 days of development, no re-parasitism occurred in pupal bees at 21 days of development. However, data on stinging of bees during larval and pupal stages indicate that "stinging only" by parasites occurred at much higher levels in early larval stages (55.2% and 34.0% at days 1 and 4 of development, respectively) than in the later pupal stages (6.7% at day 17 of development).

Table 5. Chalcid re-parasitism of leafcutting bee larvae and pupae at various stages of development during the incubation period

Treatment	Emergence (%)	Re-parasitism (%)	Cells stung (%)
Control A	86.6	-	-
Control B	83.2	-	-
21 day	85.3	0.0	0.0
17 day	73.0	5.3	6.7
14 day	58.7	9.3	17.0
10 day	55.7	10.7	18.6
07 day	53.0	14.1	17.9
04 day	42.4	8.6	34.0
01 day	21.2	8.6	55.2

iii. Field-scale research on alternative compounds for parasite control during the incubation period

A trend to increased parasitism in alfalfa leafcutting bee populations in recent years has resulted in many alfalfa seed producers using dichlorvos, the chemical traditionally utilized for parasite control, at rates exceeding the recommended rate (0.75 resin strip/1000 ft³), and using dichlorvos for extended time periods. Research has indicated that significant increases in alfalfa leafcutting bee mortality during incubation can be related to dichlorvos rate, treatment time, number of bee cells treated, bee cell position in the incubator, and incubation humidity.

In addition to the potentially damaging effects that use of dichlorvos resin strips may have on developing alfalfa leafcutting bees, recent studies by the U.S. Environmental Protection Agency (EPA) have demonstrated that dichlorvos is a carcinogen and is also implicated in nervous system damage. To eliminate what it feels is an unacceptable health risk, the EPA has included dichlorvos in the current pesticide registration reassessment initiative undertaken as part of the U.S. Food Quality Protection Act and is proposing to cancel most uses of the pesticide dichlorvos, including the use of dichlorvos resin strips. Thus there is a possibility that dichlorvos resin strips may be unavailable at some point in the near future.

Research to develop new technology for parasite control involves work in various areas of the alfalfa leafcutting bee system. Key time frames for parasite control include the spring incubation period, the summer field period, and the fall storage period. Development of successful parasite management strategies must address all of these areas of opportunity for re-parasitism. Research has indicated that a new pyrethrin aerosol compound could be efficacious for control of chalcid parasites during the spring incubation period, when alfalfa leafcutting bee cells are incubated prior to field release and are thus most susceptible to re-parasitism by chalcid parasites.

The pyrethrins are a group of natural insecticides extracted from the flowers of a *Chrysanthemum* species grown on a commercial basis, primarily in east Africa. Pyrethrins act by contact, penetrating the insect integument and paralyzing the insect nervous system. Pyrethrins also interfere with oxygen transport within insect cells. Pyrethrin synergists or activators are combined with the pyrethrins to make them more effective insecticidal agents; the synergists may actually affect the absorption of pyrethrin compounds through the insect cuticle and also prevent the insect from detoxifying the pyrethrins. The pyrethrin aerosol formulations utilized are comprised of pyrethrins and one or more pyrethrin synergists (piperonyl butoxide, n-octyl bicycloheptene dicarboximide).

The compound is released into the incubator environment as an aerosol carrying small pyrethrin particles. The most critical factor associated with use of pyrethrin aerosol for parasite control involves adequate circulation of the pyrethrin particles within the incubator. There is currently no standard leafcutting bee incubator design utilized by alfalfa seed producers; this lack of uniformity makes development and transfer of new parasite control technology difficult. For this reason, a research initiative was undertaken with the objective of designing a prototype alfalfa leafcutting bee research incubator.

The first prototype incubator to be constructed was utilized during the 1998 field season to further evaluate use of pyrethrin aerosol for chalcid parasite control. A second incubator of identical design was subsequently constructed and both incubators were utilized for chalcid parasite control research during the 1999 and 2000 field seasons. The purpose of research on the development of a prototype leafcutting bee incubator, incorporating features designed to optimize parasite control, is to provide important technical information to producers which will allow them to either construct new leafcutting bee incubators or retrofit existing incubators to a recommended set of specifications in order to enhance parasite control efforts.

The prototype leafcutting bee incubators were designed with sufficient capacity (ca 2,170 ft³) to incubate 10 million leafcutting bee cells per unit. These incubators required a ventilation system which could be adjusted in order to provide the conditions necessary to work with various compounds to be evaluated in a field-scale research system for their efficacy as parasite control agents. The construction of each incubator in a 45 foot van trailer provided a 36 foot incubation chamber and an adjacent workspace for incubator monitoring and control equipment. The ventilation system was designed around an air conditioning fan - coil whereby the air circulating in the incubators could be either pushed or pulled over incubation trays containing the leafcutting bee cells. Thus the air, whether being pushed or pulled, could be cooled. The heat source utilized for the incubators was electric baseboard heaters.

The heating and air conditioning systems were controlled by a thermostat, which employed sensors mounted at points within the leafcutting bee incubation trays and at other points in the incubation chamber in order to provide input for the thermostat to maintain programmed temperature set-points. Control of the push - pull ventilation system was attained by dampers installed on both sides of the intake and discharge points of the air conditioning fan - coil unit. Ventilation over the trays was achieved through 0.125 inch vertical slots situated directly behind each row of trays. Return or intake ventilation on the wall opposite the leafcutting bee incubation trays was attained with 0.125 inch horizontal slots that were baffled to control the direction of air movement. Air movement in the incubator is characterized in the following way:

Pull system - good mixing of air from top to bottom in the incubator opposite the alfalfa leafcutting bee incubation trays. Air movement over the incubation trays is 50 feet per minute or less.

Push system - positive and vigorous movement of air over alfalfa leafcutting bee incubation trays with even capture of return air opposite the incubation trays.

Heating/Ventilation/Air Conditioning (HVAC) calculations took into account all construction components of the incubators, as well as the heating and venting requirements to maintain the leafcutting bees at the required incubation temperature. Heat produced by the developing leafcutting bee pupae, and ventilation required for removing residual pyrethrin aerosol, was also considered in the calculations. Selection of the air conditioning unit was based on the HVAC calculation and the fan capacity was matched to the heat loss calculation of the vent circuit.

1998 field-scale parasite control research: Research was undertaken in 1998 to determine the efficacy of the pyrethrin aerosol (KN-409) for parasite control in the prototype leafcutting bee incubator; approximately 5 million leafcutting bee cells with a parasite level of 7.0% (i.e. 7.0% of bee cells infested with chalcid parasite larvae) were incubated at 30°C / 50% RH. A total of 10 pyrethrin aerosol dispensers (1 dispenser / 217 ft³) were installed on the wall of the incubation chamber, facing the leafcutting bee incubation trays. The pyrethrin aerosol dispensers were factory-calibrated to deliver 27.5 mg active ingredient / dispenser at 7.5 minute intervals. The positioning of the dispensers was undertaken in such a way that the pyrethrin aerosol plume was gently dispersed over the surface of the bee cells in the incubation trays by utilizing the "pull system" mode of air circulation within the incubation chamber.

Pyrethrin aerosol treatment continued from day 00 through day 12 of incubation; on day 13, the air circulation was changed to the "push system" mode, whereby air was moved vigorously over the trays and captured on the opposite wall of the incubator. This airing effect, in conjunction with operation of the incubator fresh air intake system, was utilized to break down the pyrethrin aerosol residue prior to adult bee emergence. Extensive sampling of bee cells was undertaken at intervals during the incubation period to determine re-parasitism of bee cells and mortality due to parasite stinging following the pyrethrin aerosol treatment period.

Combined data collected from sampling undertaken during day 12-19 of incubation is given in Table 6. These data indicate that the parasitism level of leafcutting bee cells in the incubator was reduced from 7.0% to 1.6%. Additional sampling of bee cells in incubation trays was undertaken following field deployment of the bees. Data collected from in-field sampling indicated levels of adult bee emergence, re-parasitism, and mortality due to parasite stinging that were not significantly different from data collected previously in the incubator.

**Table 6. 1998 Parasite Control Experiment
Leafcutting Bee Re-parasitism (%)**

Category / Cell contents	KN-409
Healthy LCB pupae / adults	97.4
Dead pupae re-parasitized	1.6
Dead pupae stung	1.0

1999 field-scale parasite control research: Research undertaken in 1999 involved an evaluation of pyrethrin aerosol (KN-409) for parasite control in incubator 01 and a parallel evaluation of dichlorvos for parasite control in incubator 02. In each of the two prototype leafcutting bee incubators, approximately 3.4 million bee cells with a parasite level of 2.6% were incubated at 30°C / 50% RH. Incubation trays were utilized in both the solid bottom and screen bottom mode in each of these experiments. In incubator 01, a total of 14 pyrethrin aerosol dispensers (1 dispenser / 155 ft³) were installed on the wall of the incubation chamber and parasite control treatment was undertaken as previously described. In incubator 02, 1.6 dichlorvos resin strips (0.75 resin strip / 1000 ft³) were suspended from the ceiling of the incubation chamber in such a way that the dichlorvos would be dispersed over the surface of the bee cells in the incubation trays by utilizing the "pull system" mode of air circulation within the incubation chamber. Dichlorvos treatment with the "pull system" of air circulation in place continued from day 07 through day 14 of incubation; air circulation was then changed over to the "push system", whereby air was moved vigorously over the trays in conjunction with use of the fresh air intake system until day 21 in order to break down the dichlorvos residue prior to adult bee emergence.

Extensive sampling of bee cells was undertaken in both incubators at intervals during the incubation period to determine re-parasitism of bee cells and mortality due to parasite stinging following either pyrethrin aerosol or dichlorvos treatment. Combined data collected from sampling undertaken during day 12-19 of incubation is given in Table 7. Data from the pyrethrin aerosol experiment indicate that re-parasitism of bee cells in incubator 01 occurred at levels of 1.5% (solid bottom trays) and 0.3% (screen bottom trays). Data from the dichlorvos experiment indicate that re-parasitism of bee cells in incubator 02 occurred at levels of 4.0% (solid bottom trays) and 1.8% (screen bottom trays). Clearly, in both pyrethrin aerosol and dichlorvos parasite experiments, use of screen bottom incubation trays resulted in significantly lower re-parasitism of leafcutting bee cells. This would indicate that the additional air movement through the screen bottom trays resulted in enhanced delivery of both pyrethrin aerosol and dichlorvos to bee cells in the trays.

**Table 7. 1999 Parasite Control Experiments
Leafcutting Bee Re-parasitism (%)**

Category / Cell contents	KN-409		Dichlorvos	
	solid	screen	solid	screen
Healthy LCB pupae / adults	97.8	98.5	88.0	91.0
Dead pupae re-parasitized	1.5	0.3	4.0	1.8
Dead pupae stung	0.7	1.2	8.0	7.2

During evaluation of leafcutting bee cells to determine levels of re-parasitism within the incubator in both pyrethrin aerosol and dichlorvos experiments, it was observed that in some cases, developing bee pupae were being stung and killed by parasites, but not successfully re-parasitized. As noted previously, the adult female parasite stings the leafcutting bee larva or pupa prior to oviposition on the body of the host; however, there is a possibility that either "successful re-parasitism" (i.e. stinging + oviposition) or "stinging only" will occur in some cases, perhaps in relation to the age of the larval or pupal host. Mortality due to stinging evaluated during incubation (Table 7) was found to occur at levels of 0.7% (solid bottom trays) and 1.2% (screen bottom trays) in the pyrethrin aerosol test, and at much higher levels of 8.0% (solid bottom trays) and 7.2% (screen bottom trays) in the dichlorvos test.

Additional sampling of bee cells in incubation trays was undertaken following deployment of incubation trays in field shelters. Data collected from this in-field sampling indicated levels of adult bee emergence, re-parasitism, and mortality due to parasite stinging that were not significantly different from data collected previously in the incubator. The data clearly demonstrate that, under the conditions of this experiment, pyrethrin aerosols provided a greater level of control against chalcid re-parasitism than that provided by dichlorvos.

2000 field-scale parasite control research: Research undertaken in 2000 involved an evaluation of pyrethrin aerosol (KN-409) for parasite control in incubator 01 and a parallel evaluation of pyrethrin aerosol (KN-418) for parasite control in incubator 02. Pyrethrin aerosol formulation KN-409 contains 0.975% pyrethrins, 1.950% piperonyl butoxide, and 3.210% n-octyl bicycloheptene dicarboximide, while pyrethrin aerosol formulation KN-409 contains 1.80% pyrethrins and 10.00% piperonyl butoxide.

In each of the two prototype leafcutting bee incubators, approximately 5.1 million bee cells with a parasite level of 1.7% were incubated at 30°C / 50% RH. Incubation trays were utilized in screen bottom mode only in each of these experiments. In incubator 01, a total of 14 pyrethrin aerosol dispensers (1 dispenser / 155 ft³) were installed on the wall of the incubation chamber. In incubator 02, total of 5 pyrethrin aerosol dispensers (1 dispenser / 434 ft³) were installed on the wall of the incubation chamber; in both incubators, parasite control treatment was undertaken as previously described.

Extensive sampling of bee cells was undertaken in both incubators at intervals during the incubation period to determine re-parasitism of bee cells and mortality due to parasite stinging following either KN-409 or KN-418 treatment. Combined data collected from sampling undertaken during day 12-19 of incubation is given in Table 8. Data from the KN-409 experiment indicate that re-parasitism of bee cells in incubator 01 occurred at a level of 1.0%. Data from the KN-418 experiment indicate that re-parasitism of bee cells in incubator 02 occurred at a level of 1.9%. Additional in-field sampling of bee cells indicated levels of adult bee emergence, re-parasitism, and mortality due to parasite stinging that were not significantly different from data collected previously in the incubator.

**Table 8. 2000 Parasite Control Experiments
Leafcutting Bee Re-parasitism (%)**

Category / Cell contents	KN-409	KN-418
Healthy LCB pupae / adults	98.9	97.3
Dead pupae re-parasitized	1.0	1.9
Dead pupae stung	0.1	0.8

Parasite control research in the prototype alfalfa leafcutting bee incubators has demonstrated the efficacy of pyrethrin aerosol formulations for control of chalcid parasites, the efficacy of utilizing screen bottom trays in conjunction with pyrethrin aerosol treatment, and the potential for use of pyrethrin aerosol formulations containing higher levels of pyrethrins and pyrethrin synergists. The air circulation design utilized in the prototype alfalfa leafcutting bee incubators was shown to be highly effective in maintaining uniform alfalfa leafcutting bee incubation temperatures and in delivering pyrethrin aerosol for parasite control. As well, in all of the experiments undertaken during the period 1998 through 2000, no post-emergent mortality was observed in adult male and female alfalfa leafcutting bees within the incubation trays following pyrethrin aerosol treatment, indicating that the incubator air circulation and fresh air intake system was effective in dissipating the pyrethrin aerosol residue prior to the emergence of adult bees.

iv. Research on prevention of chalcid re-parasitism in the field

Alfalfa leafcutting bee larvae are often vulnerable to parasitism by adult chalcid parasites during the field season. Adult female parasites are able to penetrate the backing of alfalfa leafcutting bee nests through small cracks left when the nest material is not compressed tightly against the nest backing. The parasites then gain access to the backs of the nest tunnels and parasitize developing larvae within the alfalfa leafcutting bee cells.

To examine parasite control in the field, a test involving modification of alfalfa leafcutting bee nest backing was undertaken during the 1997 field season. In this test, a total of 200 polystyrene laminate nests were utilized. The standard unmodified nest incorporated a layer of corrugated cardboard between nest material and wood nest backing. One hundred of these nests were left unmodified (control), while in the remaining nests each of four groups of 25 was modified using a bonded polyester fill (BPF) nest backing material either outside the cardboard layer, inside the cardboard layer, or without the cardboard as a double layer or single layer of BPF only.

As indicated in Table 9, modification of bee nests with BPF decreased re-parasitism in all treatments relative to unmodified control nests. These data clearly demonstrate the benefit of incorporating bonded polyester fill as a protective layer between the alfalfa leafcutting bee nest and the wood nest backing, in order to reduce the level of chalcid parasitism.

Table 9. Evaluation of various nest material treatments incorporating bonded polyester fill (BPF) for efficacy in control of chalcid re-parasitism *

Treatment	No. cells sampled	Pre-season level parasites (%)	Post-season level parasites (%)	Change +/- (%)
Unmodified (control)	3059	1.51	2.48	+ 0.97
Modified				
BPF outside card.	1337	1.51	0.67	- 0.84
BPF inside card.	1224	1.51	0.57	- 0.94
BPF double layer	1134	1.51	0.26	- 1.25
BPF single layer	1123	1.51	0.00	- 1.51

* BPF - Bonded polyester fill (weight 6 oz.); thickness 1" expanded / 0.125" compressed

b) Chalkbrood disease research initiatives

Annual field season trapnest surveys and Saskatchewan alfalfa leafcutting bee winter surveys undertaken during the period 1997-1998 through 2000-2001 have played an important role in the identification of sites in the province where chalkbrood disease (*Ascosphaera aggregata*) is currently present in alfalfa leafcutting bee populations and in the documentation of the level of infection. Identification of chalkbrood is a key to the control of this devastating fungal disease. The trapnest and winter surveys have also served to provide research material for the investigation of a related fungal disease (i.e. the ragged-brood species *Ascosphaera larvis*). A combined program of field and laboratory research on *A. larvis* has indicated the potential for this fungal species to become a serious problem in alfalfa leafcutting bee populations.

Work to monitor levels of both *A. aggregata* and *A. larvis* in Saskatchewan alfalfa leafcutting bee populations is ongoing. Data on the occurrence of these diseases in bee populations collected during 1997-1998 through 2000-2001 Saskatchewan alfalfa leafcutting bee winter surveys are presented in Table 10, with mean percent and associated standard deviation values given to represent the total of all populations evaluated in each survey. Data for *A. aggregata* are presented for the sporulating (S) and non-sporulating (N) forms of the disease. In larval cadavers infected with chalkbrood disease, the fungus may or may not form spores. The result is two distinctly different chalkbrood cadaver phenotypes, typically referred to as "sporulating" and "non-sporulating" cadavers.

Table 10. Incidence of disease (%) in Saskatchewan alfalfa leafcutting bee populations with data from winter surveys 1997-1998 through 2000-2001.

Year of survey	<i>A. aggregata</i> S	<i>A. aggregata</i> N	<i>A. larvis</i>
1997 - 1998	0.007 (sd 0.050)	0.022 (sd 0.112)	0.355 (sd 0.836)
1998 - 1999	0.010 (sd 0.079)	0.016 (sd 0.118)	0.354 (sd 1.062)
1999 - 2000	0.004 (sd 0.024)	0.007 (sd 0.041)	0.398 (sd 1.007)
2000 - 2001	0.030 (sd 0.119)	0.026 (sd 0.092)	0.546 (sd 1.088)

Research on the efficacy of paraformaldehyde fumigation for control of alfalfa leafcutting bee-related microflora (i.e. bacteria and fungi, including *Ascosphaera* spp.) has effectively demonstrated that under specific temperature and humidity conditions (20-25°C / 60-70% RH), fumigation of leafcutting bee nest material and bee cells with paraformaldehyde at a rate of 20g/m³ is highly efficacious for control of fungal spores in contaminated nest material (polystyrene laminate, polystyrene block, wood laminate) and on bee cell surfaces (Table 11).

Table 11. Efficacy of paraformaldehyde fumigation of leafcutting bee nest material and bee cells

Material tested	Reduction in microflora (%)
Polystyrene laminate	99.0 **
Polystyrene block	96.7 *
Wood laminate	89.3 *
Leafcutting bee cells	98.6 **

significant reduction (* $p < 0.01$, ** $p < 0.001$)

As noted previously, while the principle disease of the leafcutting bee is chalkbrood caused by the fungus *A. aggregata*, several other *Ascosphaera* species are also associated with leafcutting bees; in the past, these species have not generally caused significant mortality in leafcutting bee populations. One of the species, *A. larvis*, causes chalkbrood-like symptoms in larvae of leafcutting bees. To determine the threat *A. larvis* may pose to alfalfa leafcutting bee populations, the fungus has been monitored through surveys of leafcutting bee populations. As well, research has been undertaken in the laboratory, in collaboration with Dr. Mark Goettel (AAFC - Lethbridge), to determine susceptibility of alfalfa leafcutting bee larvae to *A. larvis*.

A. larvis was isolated from diseased insects obtained through the trapnest survey, cultured in the laboratory, identified, and re-inoculated onto healthy leafcutting bee larvae. Infected larvae exhibited similar disease symptoms as those larval cadavers from which the fungus was initially isolated. *A. larvis*-infected cadavers are easily distinguished from those infected by *A. aggregata*, since *A. larvis* sporulates on the surface of cadavers whereas *A. aggregata* sporulates beneath the cuticle surface.

Laboratory assays and field surveys indicated that *A. larvis* is a potentially virulent pathogen of alfalfa leafcutting bee larvae, capable of causing significant mortality in alfalfa leafcutting bee populations. Laboratory experiments demonstrated that a typical dose-mortality relationship exists, with increasing mortality occurring as the spore dose of *A. larvis* is increased (estimated $LD_{50} = 1.9 \times 10^5$ spores/bee). The incidence of *A. larvis* was found at levels of up to 5.62% in one of the alfalfa leafcutting bee populations monitored through the 2000-2001 Saskatchewan alfalfa leafcutting bee winter survey. At several sites in the province, both *A. larvis* and *A. aggregata* have been identified in the same alfalfa leafcutting bee populations. The threat posed to Saskatchewan alfalfa leafcutting bee populations by *A. larvis* is uncertain at this time, and it is important that levels of the disease continue to be closely monitored.

c) Research on stored product pests in alfalfa leafcutting bee populations

During the 1997-2000 field seasons, stored product pest insect specimens (larvae and adults) were collected from leafcutting bee trapnests, trapnest cell samples, and leafcutting bee cell samples submitted by producers. Where necessary, insect larvae were reared to the adult stage in the laboratory. All specimens collected have been identified to genus and species level. A list of the stored product pest insect species found over the past four years in association with alfalfa leafcutting bee populations is given in Table 12.

Table 12. Stored product pest insect species found in alfalfa leafcutting bee populations during the 1997 - 2000 field seasons

Common name	Scientific name
Black carpet beetle	<i>Attagenus unicolor</i> (Braham)
Confused flour beetle	<i>Tribolium confusum</i> du Val
Driedfruit moth	<i>Vitula edmandsae serratilineella</i> Ragonot
Larder beetle	<i>Dermestes lardarius</i> Linnaeus
Rusty grain beetle	<i>Cryptolestes ferrugineus</i> (Stephens)
White-marked spider beetle	<i>Ptinus fur</i> Linné
Yellow mealworm	<i>Tenebrio molitor</i> Linnaeus

While the two most common stored product pest insect species collected from alfalfa leafcutting bee populations during the 1997 field season were the driedfruit moth and the black carpet beetle, the black carpet beetle and the confused flour beetle were most commonly observed during the 1998 field season. In the 1999 and 2000 field seasons, the driedfruit moth and the black carpet beetle were once again the two most common stored product pest insect species observed. An overview of the relative incidence of all stored product pest insect species identified over the past four years is given in Table 13.

The driedfruit moth (*Vitula edmandsae serratilineella*) is most commonly seen in its larval form when cells are removed from nest material. The larvae may chew through polystyrene nest material and destroy bee cells in their search for pollen and nectar prior to overwintering and emergence in spring as adult moths. Larvae of the black carpet beetle (*Attagenus unicolor*) and the confused flour beetle (*Tribolium confusum*) may also damage nest material and bee cells, killing bee larvae as they search for pollen. These beetles are capable of feeding at low temperatures and producing more than one generation per year.

Control of stored product pest insects involves adequate breaking and tumbling of bee cells, and utilization of proper bee cell storage techniques, especially during winter storage. Since many stored product pest insect species are active at relatively low temperatures, it is recommended that alfalfa leafcutting bee cells be held in the 4-10°C range for the period between cell harvest in the fall and bee cell incubation in the spring. Research has also indicated that treatment of bee cells with pyrethrin aerosols for chalcid parasite control in the incubator will control stored product pest insect species active in the incubator during the early part of the of incubation period.

Table 13. Stored product pest incidence in alfalfa leafcutting bee populations over four year period 1997-2000

Trapnest number *	BCB				CFB				DFM				DLB				WSB			
	97	98	99	00	97	98	99	00	97	98	99	00	97	98	99	00	97	98	99	00
01				+	+															+
23 (02)				(+)	+				+		(+)									
17 (03)	+	+							+		(+)									
03 (04)		+		(+)	+															
20 (05)	+			(+)	+				+				+						+	
09 (06)	+			+					+		(+)									
05 (07)	+								+	+	(+)									
21	+	+							+	+										
(08)			+	+																
10	+	+			+	+														
12 (11)						+			+											
13	+								+											
08	+	+																		
14 (12)	+	+							+				+							
25 (13)	+																			
07 (14)		+			+	+							+							
18 (15)	+			+	+				+											
04 (16)								+												
16 (17)	+		+	+	+				+											
26 (18)	+	+			+				+			+								

+

specimen(s) found in trapnest / harvested cells

(+)

damage to bee cells / nest material by this species observed (no larvae found in trapnest)

BCB Black carpet beetle

CFB Confused flour beetle

DFM Driedfruit moth

DLB Dermestid (Larder) beetle

WSB White-marked spider beetle

Yellow mealworm (YMW) was noted in trapnest 18 (15) in the 1997 field season and in trapnest 07 (14) in the 1998 field season; as well, rusty grain beetle (RGB) was noted in trapnests 18 (15) and 04 (16) in the 2000 field season.

* Note: new trapnest numbering system incorporated in the 1999 field season

d) 1997-2000 alfalfa leafcutting bee trapnest research summary

Monitoring of parasites, predators, and diseases in alfalfa leafcutting bee populations has been undertaken through placement of specially constructed trapnests in alfalfa seed fields throughout Saskatchewan during the 1997-2000 field seasons. The purpose of the trapnest research program is to assist in the identification of chalkbrood disease, chalcid parasitism, and other insect pest problems as they occur. During each of the past four field seasons, 18-20 trapnests were placed in alfalfa leafcutting bee shelters at locations in various alfalfa seed producing areas of the province. The trapnests remained in the field from June through September; alfalfa leafcutting bee cells were harvested in early October, and the contents of alfalfa leafcutting bee cell samples from each trapnest were evaluated in late October and early November. An overview of Saskatchewan trapnest project data from the alfalfa leafcutting bee (*Megachile rotundata*) cells collected in the trapnests over the four year period is given in Table 14.

Table 14. 1997-2000 Saskatchewan trapnest data summary (expressed as % of total cells)

Category / Cell contents	1997	1998	1999	2000
HEALTHY PREPUPAE	74.82	69.45	74.77	73.98
DEAD LARVAE/PREPUPAE				
Discoloured	3.62	6.65	5.19	3.02
Mouldy	0.16	0.57	0.55	0.24
Sporulating chalkbrood	0.06	0.41	0.04	0.49
Non-sporulating chalkbrood	0.16	0.20	0.12	0.24
Other <i>Ascosphaera</i> spp.	0.45	0.22	0.30	0.49
MOULDY CELLS				
Pollen Mould	2.23	2.05	5.52	7.39
Pollen <i>Ascosphaera</i> spp.	0.68	0.38	2.04	0.14
PARASITES	4.55	5.38	1.93	3.46
SECOND GENERATION	0.11	0.19	0.28	0.71
POLLEN BALLS	12.18	14.53	9.26	9.85
NO. CELLS ANALYSED	23,108	9,721	9,751	9,518

Analysis of alfalfa leafcutting bee cells (*Megachile rotundata*) collected annually in the trapnests indicated that chalkbrood disease (*Ascosphaera aggregata*) in the sporulating form was present at levels of 0.04-0.49% overall during the period 1997-2000. The non-sporulating form of *A. aggregata* was identified at levels ranging from 0.12-0.24% over the same period. The related pathogen *Ascosphaera larvis* was present at levels ranging from 0.22-0.49% overall.

Analysis of native leafcutting bee cells (*Megachile relativa*) collected annually in the trapnests (range 26-218 cells annually over the four year period) indicated that *A. aggregata* was not present in the native leafcutting bee species; however, in the 1998 field season, *A. larvis* was identified at a level of 0.46% overall in native leafcutting bee cells evaluated.

Analysis of data collected from the 1997-2000 trapnest surveys has indicated the presence of chalcid parasites (*Pteromalus venustus*) at overall levels of 1.93-5.38%. Trapnest data on chalkbrood and parasite levels in leafcutting bee populations at locations throughout the province are utilized to determine the current status of these problems and to guide research initiatives aimed at development of management strategies.

**e) Alfalfa leafcutting bee winter survey summary
(1997-1998 through 2000-2001)**

The ongoing 2000-2001 Saskatchewan alfalfa leafcutting bee winter survey has included bee cell samples from a total of 94 populations received from alfalfa seed producers to date and analyzed during the period December 12, 2000 through March 23, 2001. In the 2000-2001 survey, Saskatchewan leafcutting bee populations were evaluated for the presence of chalkbrood disease (*Ascosphaera aggregata*), chalcid parasites (*Pteromalus venustus*), larval moulds, pollen moulds, and numerous other important factors affecting bee populations. Data collected through evaluation of samples was returned to individual producer-cooperators participating in the survey; an overview of data from the 2000-2001 survey is given in Table 15.

Table 15. 2000-2001 Saskatchewan alfalfa leafcutting bee survey data summary

Category / Cell contents	Statistic (%)			
	Mean	S.dev.	Max.	Min.
HEALTHY PREPUPAE	80.01	5.67	90.31	64.10
DEAD LARVAE/PREPUPAE				
Discoloured	2.23	1.60	10.53	0.42
Mouldy	0.20	0.28	1.71	0.00
Sporulating chalkbrood	0.030	0.119	0.759	0.00
Non-sporulating chalkbrood	0.026	0.092	0.525	0.00
Other <i>Ascosphaera</i> spp.	0.546	1.088	5.620	0.00
MOULDY CELLS				
Pollen Mould	3.16	3.48	14.52	0.00
Pollen <i>Ascosphaera</i> spp.	0.59	0.76	3.31	0.00
PARASITES	0.55	1.06	5.10	0.00
SECOND GENERATION	0.61	1.63	13.09	0.00
POLLEN BALLS	12.06	4.37	26.79	3.83
NO. CELLS ANALYSED	724	49	897	626
LIVE COUNT/LB.	4386	396	5299	3437
LIVE COUNT/KG.	9655	872	11669	7568

Chalkbrood disease (*Ascosphaera aggregata*) has been identified in several of the alfalfa leafcutting bee populations evaluated to date in the 2000-2001 winter survey. In the sporulating form, *A. aggregata* was identified in these populations at levels ranging from 0.134% to 0.759%. The non-sporulating form of *A. aggregata* was identified in these populations at levels of 0.134% to 0.525%. The mean occurrence of *A. aggregata* in the 2000-2001 winter survey overall was 0.030% sporulating chalkbrood and 0.026% non-sporulating chalkbrood.

Mean occurrence of other *Ascosphaera* species in the larval category was 0.546%. One of these species (*Ascosphaera larvis*) was found at a level of 5.620% in one alfalfa leafcutting bee population. As noted previously in this research report, *A. larvis* is a fungal species which is not classic chalkbrood (i.e. *A. aggregata*), but which occurs sporadically in some alfalfa leafcutting bee populations. Mean occurrence of *Ascosphaera* species on pollen balls was 0.59%. The category entitled "pollen *Ascosphaera* spp." refers to native *Ascosphaera* fungal species (i.e. *Ascosphaera pollenicola*) and related species found growing on pollen collected for use in cell provisioning by the female alfalfa leafcutting bee.

A brief overview of other data collected to date in the 2000-2001 Saskatchewan alfalfa leafcutting bee survey indicates that the mean level of healthy prepupae is 80.01%. Larval and pollen moulds, at 0.20% and 3.16% respectively, were found at fairly low levels despite the cool and wet conditions prevalent in many alfalfa seed production areas during the 2000 field season. Other categories included in the survey data summary are discoloured dead larvae - 2.23%, second generation (i.e. bee larvae exhibiting the trait of multivoltinism, whereby the normal overwintering state of diapause is not entered) - 0.61%, and pollen balls - 12.06%.

The overall level of chalkbrood disease (*A. aggregata*) documented in the annual winter alfalfa leafcutting bee surveys has remained relatively low over the period 1997-2000, ranging from 0.01-0.03% for sporulating chalkbrood, and from 0.01-0.03% for non-sporulating chalkbrood as well. The overall level of *A. larvis* in these surveys has ranged from 0.35-0.55% over the same four year period. The mean level of chalcid parasites in current year's survey is 0.55%, down significantly from 1.11%, 2.02%, and 1.26% found in 1997-1998, 1998-1999, and 1999-2000 surveys, respectively (Table 16).

Saskatchewan alfalfa leafcutting bee winter survey data on chalcid parasite and chalkbrood disease levels in alfalfa leafcutting bee populations has been utilized to determine the current status of these problems, and to guide research initiatives aimed at the development and application of management strategies. As the acreage devoted to alfalfa seed production has risen dramatically over the past five years in Saskatchewan, incidence of chalkbrood disease is a constant concern to producers involved in the industry. In populations where chalkbrood disease has been identified, additional alfalfa leafcutting bee sampling is undertaken as part of a comprehensive approach to control of the disease. This approach involves accurately determining the level of the disease problem, identifying the source of *A. aggregata* inoculum if possible, and utilizing appropriate methods, including paraformaldehyde fumigation, for control of chalkbrood spores in alfalfa leafcutting bee nest material and bee cells.

Table 16. 1997-1998 through 2000-2001 Saskatchewan alfalfa leafcutting bee survey data summary (expressed as % of total cells)

Category / Cell contents	1997- 1998	1998- 1999	1999- 2000	2000- 2001
HEALTHY PREPUPAE	85.01	82.67	85.54	80.01
DEAD LARVAE/PREPUPAE				
Discoloured	1.80	2.89	2.97	2.23
Mouldy	0.10	0.40	0.37	0.20
Sporulating chalkbrood	0.01	0.01	0.01	0.03
Non-sporulating chalkbrood	0.02	0.02	0.01	0.03
Other <i>Ascosphaera</i> spp.	0.36	0.35	0.40	0.55
MOULDY CELLS				
Pollen Mould	2.37	2.69	2.71	3.16
Pollen <i>Ascosphaera</i> spp.	n/a	0.62	0.67	0.59
PARASITES	1.11	2.02	1.26	0.55
SECOND GENERATION	0.21	0.27	0.32	0.61
POLLEN BALLS	9.11	8.05	5.75	12.06
LIVE COUNT / LB	4327	4159	4342	4389
LIVE COUNT / KG	9534	9159	9560	9655

B. Technical report

4. Indications for further research

Problems with chalcid parasite (*Pteromalus venustus*), chalkbrood disease (*Ascosphaera aggregata*), and stored product pest infestations in Saskatchewan alfalfa leafcutting bee populations are ongoing. As noted previously in this report, with the acreage devoted to alfalfa seed production having risen dramatically over the past five years in Saskatchewan, the incidence of chalkbrood disease and the control of chalcid parasites continue to be major concerns for producers involved in the industry. The quality of alfalfa leafcutting bee populations is dependent upon annual insect pest and disease surveys, continuing development of management strategies, and transfer of appropriate technology to alfalfa seed producers. Continuing parasite and disease research, and extension of information to producers, is critical in achieving the goal of ensuring high quality alfalfa leafcutting bee populations in Saskatchewan. This will serve to enhance alfalfa seed production in the province, and increase the value of Saskatchewan alfalfa leafcutting bees in export markets.

C. Personnel**AFIF-Hub Project 19970004:**

Parasite, predator, and disease management
strategies in alfalfa leafcutting bees

Lead Researcher, AFIF-Hub Project

D.W. Goerzen - Biologist / Research Director
Saskatchewan Alfalfa Seed Producers Association

Research Technical Assistance

1. Diane Davis - research technician
2. Ivana Fellnerova - research technician

D. Equipment rental

An agreement was entered into between the AFIF-Hub Project 19970004 "Parasite, predator, and disease management strategies in alfalfa leafcutting bees" and the Saskatchewan Alfalfa Seed Producers Association (SASPA) to facilitate the annual rental of two prototype alfalfa leafcutting bee research incubators utilizing project funds designated for equipment rental purposes. These prototype alfalfa leafcutting bee research incubators were designed and constructed by SASPA specifically for the purpose of supporting alfalfa leafcutting bee parasite control research initiatives undertaken as part of this project.

E. Project photos

No project-related photographs are available for inclusion in this research project final report.

F. AFIF acknowledgement

Acknowledgement of funding received from the Agri-Food Innovation Fund has been included in all extension publications, conference proceedings, and newsletter articles where material relating to the AFIF-Hub Project 19970004 "Parasite, predator, and disease management strategies in alfalfa leafcutting bees" has been published, and at all forums including conferences, meetings, and producer field days where material relating to the research project has been discussed.

G. Technology Transfer

AFIF-Hub Project 19970004:

Parasite, predator, and disease management strategies in alfalfa leafcutting bees

Technology Transfer

April 01, 1997 - March 31, 2001

1. Extension Publications

Goerzen, D.W. Alfalfa leafcutting bee parasite control with pyrethrin aerosols. *Saskatchewan Alfalfa Seed Producers Association Extension Publ.* 2001-03 (ISBN 0-88656-732-7); 2 pages. (March, 2001 - in press).

Goerzen, D.W. Chalkbrood disease in alfalfa leafcutting bee populations. *Saskatchewan Alfalfa Seed Producers Association Extension Publ.* 2001-02 (ISBN 0-88656-728-9); 2 pages. (March, 2001 - in press).

Goerzen, D.W. New alfalfa leafcutting bee incubator design for optimizing parasite control. *Saskatchewan Alfalfa Seed Producers Association Extension Publ.* 2001-01 (ISBN 0-88656-730-0); 2 pages. (February, 2001).

Goerzen, D.W. Stored product pests in Saskatchewan alfalfa leafcutting bee populations. *Saskatchewan Alfalfa Seed Producers Association Extension Publ.* 2000-02 (ISBN 0-88656-720-3); 2 pages. (November, 2000).

Goerzen, D.W. editor / Alfalfa leafcutting bee management and alfalfa seed production manual. *Saskatchewan Alfalfa Seed Producers Association Extension Publ.* 2000-01 (ISBN 0-88656-694-0); 925 pages. (July, 2000).

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2. Publications in Refereed Scientific Journals

Soroka, J.J., D.W. Goerzen, K.C. Falk, and K.E. Bett. 2001. Alfalfa leafcutting bee (Hymenoptera: Megachilidae) pollination of oilseed rape (*Brassica napus* L.) under isolation tents for hybrid seed production. *Can. J. Plant Sci.* 81: 199-204.

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Lu, R., D.W. Goerzen, and G.H. Rank. 1996. Use of RAPD analysis to distinguish immigrant bee species within populations of the alfalfa leafcutting bee, *Megachile rotundata* (Fabricius) (Hymenoptera: Megachilidae). *Bee Sci.* 4: 30-37.

3. Papers in Published Conference Proceedings

Goerzen, D.W. 2001. Alfalfa leafcutting bee parasite, predator, and disease control research. *Proc. SASPA 2001 Alfalfa Seed Conference, Saskatoon, SASKATCHEWAN; (January, 2001) pp. 43-50.*

Goerzen, D.W. 2000. Parasite, predator, and disease management strategies in alfalfa leafcutting bees. *Proc. 2000 Agri-Food Innovation Fund Conference, Regina, SASKATCHEWAN; (November, 2000) 2 p.*

Goerzen, D.W. 2000. Research on parasite and disease control in alfalfa leafcutting bee populations. *Proc. 18th Canadian Alfalfa Seed Conference, Edmonton, ALBERTA; (January, 2000) pp. 13-20.*

Goerzen, D.W. 1999. Recent research initiatives for parasite control in alfalfa leafcutting bee populations. *Proc. 17th Canadian Alfalfa Seed Conference, Winnipeg, MANITOBA; (January, 1999) pp. 30-41.*

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Goerzen, D.W. 2000. Leafcutting bee parasite and disease research update - 2000. *Report of the Sask. Advisory Council on Forage Crops (Insects and Diseases Committee Report). November, 2000.*

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Goerzen, D.W. 1998. Parasite and disease levels in Saskatchewan alfalfa leafcutting bee populations. Minutes of the Western Committee on Crop Protection (Report to the Expert Committee on Forage Crops). October, 1998.

Goerzen, D.W. 1997. Parasite and disease levels in Saskatchewan alfalfa leafcutting bee populations. Minutes of the Sask. Advisory Council on Crop Protection (Saskatchewan Advisory Sub-Council on Insect Control). November, 1997. p.14.

Goerzen, D.W. 1997. Leafcutting bee research update - 1997. Report of the Sask. Advisory Council on Forage Crops (Insects and Diseases Sub-Committee). November, 1997.

Goerzen, D.W. 1997. Parasite and disease levels in Saskatchewan alfalfa leafcutting bee populations. Minutes of the Western Committee on Crop Protection (Report to the Expert Committee on Forage Crops). October, 1997.

5. Conference Presentations

- a) SASPA 2001 Alfalfa Seed Conference. January 11-12th, 2001; Saskatoon, SASKATCHEWAN.
- b) 2000 Agri-Food Innovation Fund Conference November 7-8th, 2000 Regina, SASKATCHEWAN.
- c) 18th Canadian Alfalfa Seed Conference. January 18-20th, 2000; Edmonton, ALBERTA.
- d) 17th Canadian Alfalfa Seed Conference. January 24-26th, 1999; Winnipeg, MANITOBA.
- e) 16th Canadian Alfalfa Seed Conference. January 15-17th, 1998; Saskatoon, SASKATCHEWAN.
- f) 28th Northwest Alfalfa Seed Conference. January 27-29, 1997; Las Cruces, NEW MEXICO.
- g) 15th Canadian Alfalfa Seed Conference. January 9-11th, 1997; Banff, ALBERTA.

6. Presentations to Producer Groups

- a) 29th Saskatchewan Alfalfa Seed Producers Association Annual Meeting, January 11-12th, 2001; Saskatoon, Saskatchewan.
- b) Saskatchewan Alfalfa Seed Producers Association Annual Field Day, July 20th, 2000; Arborfield, Saskatchewan.
- c) 28th Saskatchewan Alfalfa Seed Producers Association Annual Meeting, January 13-14th, 2000; Saskatoon, Saskatchewan.
- d) Saskatchewan Alfalfa Seed Producers Association Annual Field Day, July 15th, 1999; Zenon Park, Saskatchewan.
- e) 27th Saskatchewan Alfalfa Seed Producers Association Annual Meeting, January 11-12th, 1999; Saskatoon, Saskatchewan.
- f) Saskatchewan Alfalfa Seed Producers Association Annual Field Day, July 16th, 1998; AAFC / Melfort Research Farm, Melfort, Saskatchewan.
- g) 26th Saskatchewan Alfalfa Seed Producers Association Annual Meeting, January 15th, 1998; Saskatoon, Saskatchewan.

- h) Saskatchewan Alfalfa Seed Producers Association Annual Field Day, July 17th, 1997; Arborfield, Saskatchewan.
- i) Manitoba Forage Seed Association Annual Meeting, January 19-21st, 1997; Winnipeg, Manitoba.
- j) 25th Saskatchewan Alfalfa Seed Producers Association Annual Meeting, January 3-4th, 1997; Saskatoon, Saskatchewan.

7. Extension Publications on the AgriCarta Web-site in the Crops / Forage - Alfalfa Seed section

- a) Alfalfa leafcutting bee mortality associated with use of dichlorvos for chalcid parasite control.
- b) Alfalfa leafcutting bee moulds and methods for their control.
- c) Alfalfa seed and leafcutting bee production in Saskatchewan.
- d) Chalkbrood control in alfalfa leafcutting bees.
- e) Microorganisms associated with the alfalfa leafcutting bee and their potential impact on producer health.
- f) Paraformaldehyde fumigation for surface decontamination of alfalfa leafcutting bee cells.
- g) Paraformaldehyde fumigation of alfalfa leafcutting bee nest material.

8. Newsletter Contributions

2000

- a) SASPA Newsletter, Vol.13, No.3. Stored product pests in alfalfa leafcutting bee populations. November, 2000; p.13.
- b) SASPA Newsletter, Vol.13, No.2. Chalkbrood control in alfalfa leafcutting bees. May, 2000; pp.11-14.
- c) SASPA Newsletter, Vol.13, No.1. Research update on parasite and disease control in alfalfa leafcutting bee populations. February, 2000; pp.29-36.
- d) Forage Seed News / Manitoba Forage Seed Association, Vol. 7, No.1. Research on parasite and disease control in alfalfa leafcutting bee populations. January, 2000; pp.35-40.

1999

- a) SASPA Newsletter, Vol.12, No.2. Chalkbrood control in alfalfa leafcutting bees. April, 1999; pp. 11-14.
- b) Forage Seed News / Manitoba Forage Seed Association, Vol.6, No.2. Chalkbrood control in alfalfa leafcutting bees. Spring, 1999; pp. 13-16.
- c) SASPA Newsletter, Vol.12, No.1. Recent research initiatives for parasite control in alfalfa leafcutting bee populations. February, 1999; pp. 17-24.

1998

- a) Irrigated Alfalfa Seed Producers - Alberta Newsletter. Leafcutting bee foliar moulds and producer health. November, 1998; p. 18.
- b) Forage Seed News / Manitoba Forage Seed Association, Vol.4, No.3. SASPA prototype leafcutting bee incubator research update. Fall, 1998; p. 7.
- c) Forage Seed News / Manitoba Forage Seed Association, Vol.4, No.3. Agri-Food Innovation Fund projects relating to alfalfa seed production. Fall, 1998. p. 6.
- d) Irrigated Alfalfa Seed Producers - Alberta Newsletter. Parasite control during the field season using bonded polyester fill nest backing material. July, 1998; p. 2.
- e) Forage Seed News / Manitoba Forage Seed Association, Vol.4, No.2. Stored product pest research in Saskatchewan alfalfa leafcutting bee populations. Summer, 1998; p. 25.
- f) Forage Seed News / Manitoba Forage Seed Association, Vol.4, No.2. Parasite control during the field season using bonded polyester fill nest backing material. Summer, 1998; p. 10.
- g) SASPA Newsletter, Vol.11, No.1. Chalkbrood control in alfalfa leafcutting bees. April, 1998; pp. 15-18.
- h) SASPA Newsletter, Vol.11, No.1. Research on stored product pests in alfalfa leafcutting bee populations in Saskatchewan. April, 1998; p. 7.

- i) SASPA Newsletter, Vol.11, No.1. Research on control of parasites during the field season utilizing bonded polyester fill nest backing material. April, 1998; p. 3.

1997

- a) SASPA Newsletter, Vol.10, No.3. Agri-Food Innovation Fund projects relating to alfalfa seed production. November, 1997. p. 4.
b) SASPA Newsletter, Vol.10, No.2. Dichlorvos (Vapona[®]) update. May, 1997; p. 3.
c) Forage Seed News / Manitoba Forage Seed Association, Vol.3, No.1. Parasite control research in alfalfa leafcutting bee populations. March, 1997; 8 pp.
d) SASPA Newsletter, Vol.10, No.1. Research on the control of chalcid parasites in alfalfa leafcutting bee populations. February, 1997; pp. 22-29.
e) SASPA Newsletter, Vol.10, No.1. Chalkbrood control in alfalfa leafcutting bees. February, 1997; pp. vi-ix.
f) SASPA Newsletter, Vol.10, No.1. Parasite control research update. February, 1997; pp. iv-v.

I. Deliverables for evaluation

The stated requirements for the research project period 1997-1998 through 2000-2001 involved work relating to the monitoring of alfalfa leafcutting bee populations and research to further develop pest management strategies through laboratory and field-scale research initiatives. Research responsibilities in the area of alfalfa leafcutting bee population monitoring have been addressed through work undertaken in the 1997-2000 alfalfa leafcutting bee trapnest surveys and in the 1997-1998 through 2000-2001 Saskatchewan alfalfa leafcutting bee winter surveys. Research requirements pertaining to the development of pest management strategies have been undertaken through continuing evaluation of potential chalcid parasite control compounds in the laboratory and through evaluation of a pyrethrin aerosol formulation as an alternative parasite control compound in field-scale trials utilizing the newly-designed prototype alfalfa leafcutting bee incubators. In addition to chalcid parasite and chalkbrood disease research, monitoring of stored product pest insect species present in Saskatchewan alfalfa leafcutting bee populations was undertaken during the 1997-2000 field seasons.

Annual alfalfa leafcutting bee trapnest surveys and comprehensive alfalfa leafcutting bee winter surveys have delivered to alfalfa seed producers accurate and timely data on the status of chalkbrood disease and chalcid parasites in Saskatchewan alfalfa leafcutting bee populations. Producers have also been made aware of the occurrence of stored product pest insect species in their leafcutting bee populations. Management strategies for dealing with chalkbrood infestations and parasite problems have been made available through direct transfer of technology from this project to alfalfa seed producers. As well, parasite control research undertaken in the prototype alfalfa leafcutting bee incubators has demonstrated the efficacy of pyrethrin aerosol formulations for control of chalcid parasites, the efficacy of utilizing screen bottom trays in conjunction with pyrethrin aerosol treatment for parasite control, and the potential for use of pyrethrin aerosol formulations containing higher levels of pyrethrins and pyrethrin synergists. The air circulation design utilized in the prototype alfalfa leafcutting bee incubators has demonstrated highly effective technology for maintaining uniform leafcutting bee incubation temperatures, for delivering pyrethrin aerosol, and for dissipating pyrethrin aerosol residue.

As noted in the technology transfer section of this alfalfa leafcutting bee research project final report, extension publications in the following areas have been recently published or are currently in press:

- alfalfa leafcutting bee parasite control with pyrethrin aerosols
- chalkbrood disease in alfalfa leafcutting bee populations
- new alfalfa leafcutting bee incubator design for optimizing parasite control
- stored product pests in Saskatchewan alfalfa leafcutting bee populations

These extension publications will be made available to Saskatchewan alfalfa seed producers through distribution with alfalfa seed industry newsletters and through inclusion in the Saskatchewan Alfalfa Seed Producers Association alfalfa leafcutting bee management and alfalfa seed production manual.